THE DIPHTHEROID BACILLUS OF PREISZ-NOCARD FROM EQUINE, BOVINE, AND OVINE ABSCESSES *

ULCERATIVE LYMPHANGITIS AND CASEOUS LYMPHADENITIS

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With Fisher[1] one of us (H.) has described a series of abscesses in 11 horses and 1 calf from all but one of which we obtained pure primary cultures of a diphtheroid bacillus identical with an organism recovered by us from caseous lymphadenitis in sheep. This organism appears to be the same as that described by certain American and European writers as the bacillus of Preisz-Nocard, which causes a disease among horses generally known as ulcerative lymphangitis. There is some question as to whether all the cases we observed were truly lymphangitic; at any rate one was very suggestive of it, and the deep origin of many of the abscesses seemed to indicate a localization in certain lymphatic nodes. We wish now to describe some of the bacteriologic aspects of our work, which is the first to show the occurrence of equine and bovine infections due to B. Preisz-Nocard in the United States.

MORPHOLOGY

Smears from the pus of the abscesses were stained by Ziehl-Nielsen's carbolfuchsin, followed by 10% HCl for acid-fast organisms, and by Gram's method. Gram's stain showed in each case typical gram-positive cocco-, diplo-, or diphtheroid bacilli of pleomorphic character, frequently in palisade arrangement, at other times in irregular masses.

The pus, diluted in glucose broth, was streaked out therefrom on rabbit- or horse-blood agar plates and tubes. Gram stains were made of cultures incubated overnight at 37 C., to ascertain the purity of the original culture. However, isolated colonies were always picked up for further study, and after confirming the positive reaction to Gram's stain and the non-acidfastness in 24-hour cultures on blood agar, the various morphologic and physiologic properties of the bacillus were determined.

Plain agar and glycerin agar proved unsuitable for continued cultivation. On agar containing 7.5% rabbit or horse blood growth is moderate after 24 hours at 37 C., freshly isolated cultures growing less vigorously than older ones. Isolated colonies are circular, umbonate, ochraceous or cretaceous, and opaque. On incubation for several days they may reach a size of 8 to 10 mm., and show formation of concentric rings about the elevated papilliform center.

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[1] Jour. Am. Vet. Med. Assn., 1915, 48, p. 18.

When touched with the needle the growth is peculiarly friable, the colony breaking apart readily. Growth is best at 37 C., but longer incubation at 20 C. in the dark is successful. Hemolysis of plated blood agar is marked in the absence of fermentable carbohydrates.

On Löffler's blood serum, an excellent medium, for which equine or bovine serum serves equally well, growth is similar to that on blood agar, with more suggestion of a yellowish insoluble pigment on Löffler's blood serum, particularly that of bovine origin. (Our blood serum consisted of 3 parts horse serum with 1 part beef broth containing 1% Witte's peptone, 0.5% NaCl, 1% dextrose, and 5% glycerin, the mixture being filtered through a Berkefeld filter, tubed, slanted, and sterilized in the autoclave at 10 pounds pressure for 30 minutes. Several strains of B. diphtheriae grown upon media prepared in this way and on media prepared by the usual fractional sterilization in the Arnold have shown no difference in growth or morphology.) On Löffler's blood serum the organism is diphtheroid, with a tendency, as with B. diphtheriae, tho less marked, toward slender, clubbed forms showing metachromatic granules with Löffler's methylene blue. On this medium the length was from 1.0 to 1.6 microns, and the width, from 0.4 to 0.5 micron.

Gelatin at 37 C. affords a moderate granular growth, but when placed in the ice-chest for an hour or so or at 20 C. it becomes hard. Tests were made daily for liquefaction for 5 days.

One-percent glucose broth yields a scanty granular deposit with slight or no pellicle-formation or clouding of medium. Broth containing no glucose develops a pellicle similar to that of B. diphtheriae with analogous sinking and formation of granular sediment. The elaboration of a soluble toxin and hemolysin in this medium will be discussed later.

Anaerobic growth in dextrose broth under hydrocarbon oil proceeds as without oil; these organisms are therefore facultative anaerobes, but shake cultures in deep dextrose agar showed their preference for aerobic conditions. The organisms were lacking in motility. No encapsulated forms were found.

These organisms in their growth on agar containing gentian violet, according to the method of Churchman,[2] were distinctly inhibited by a concentration of 1 : 100,000.

Of significance with relation to Hodgkin's disease in man is the fact that antiformin dissolved cultures of 6 strains of B. Preisz-Nocard, whereas the organisms observed by Fraenkel and Much[3] and cultivated by Bunting and Yates[4] and others from this disease, tho non-acidfast, are said to be frankly resistant to antiformin.

Cultures 10 days old on blood agar were suspended each in 10 c.c. of 0.85% NaCl, equal parts of antiformin added, and the mixtures left at 36 C. for 2 hours. They were then centrifugated, the supernatant fluid decanted, refilled with salt solution, again centrifugated and again the clear fluid decanted. Slides stained by the Ziehl-Nielsen and Gram methods failed to show anything but debris, while plants on blood agar remained barren. Untreated emulsions, however, showed the usual gram-positive cocco-bacilli and cultures on blood

[2] Jour. Exper. Med., 1912, 16, p. 221.
[3] Ztschr. f. Hyg. u. Infektionskrankh., 1910, 67, p. 159.
[4] Arch. Int. Med., 1913, 12, p. 236.

agar were successful. The experiment was repeated with a mixture of cultures 3 days old, with similar results at the end of 1 hour's treatment.

From these experiments it would seem that the organism in Hodgkin's disease is dissimilar.

Fermentation Reactions.—These reactions, tho often irregular in freshly isolated cultures, necessitated provisional identification by observation of growth on solid media, morphology, and tinctorial reactions. Each of our cultures was acclimatized to blood agar by weekly transplantation and, after 24 hours' incubation at 37 C., maintenance in diffuse light at about 20 C. A few transfers sufficed to render the reactions in Hiss's serum water media (carbohydrate 1%) constant. Dextrose (Eimer and Amend), levulose (Kahlbaum), and maltose (Merck), were fermented with formation of acid; with dextrose and maltose coagulation occurred earlier for all strains than with levulose. No gas-formation was found. Lactose (Eimer and Amend), saccharose (Eimer and Amend), raffinose (Eimer and Amend), dextrin (Merck), inulin (Eimer and Amend), glycerol (Braun-Knecht-Heimann), mannite (Eimer and Amend), and dulcite (Eimer and Amend), were not acidified in 5 days at 37 C. Litmus milk was unchanged in appearance.

These media were specially prepared, care being taken to prevent hydrolysis by overheating. Subplants were made from each tube, after 24 hours at 37 C., on blood agar to confirm the success of implantation and freedom from contamination. We emphasize this step in technic as a check against error in fermentation tests; for example, failure of proliferation, or extraneous organisms.

Table 1 shows that B. Preisz-Nocard in its fermentation reactions most resembles B. flavidus. The former may be differentiated by its failure to produce acid in glycerol.

TABLE 1

FERMENTATION REACTIONS OF DIPHTHEROID BACILLI

	Dextrose	Maltose	Glycerol	Saccharose	Dextrin	Mannite
Urethral diphtheroids (9)	+	+	..	+	+	—
B. diphtheriae (5) (6) (7) (8) (9)	+	+	+	—	+	—
B. xerosis (5) (6) (7) (8) (9)	+	+	±	+	—	—
B. Hoagii (8)	+	—	—	+	—	—
B. flavidus (8)	+	+	+	—	—	—
B. Preisz-Nocard	+	+	—	—	—	—
B. coryzae segmentosus (9)	+	—	—	—	—	—
B. Hofmanni (5) (6) (7) (8) (9)	—	—	—	—	—	—

The sign + means acid formation; — means no acid formation; the figures following the names of the organisms refer to references in footnotes.

Dunham's peptone salt solution gave a negative nitroso-indol reaction with concentrated H_2SO_4, and no free indol with $NaNO_2$. Nitrate broth, while allow-

[5] Jour. Med. Research, 1904, 7, p. 475.
[6] Jour. Hyg., 1906, 6, p. 286.
[7] Jour. Med. Research, 1907, 17, p. 277.
[8] Jour. Infect. Dis., 1912, 11, p. 253.
[9] Jour. Path. and Bacteriol., 1913, 18, p. 75.

ing faint development, showed no reduction to nitrites at the end of 5 days' incubation with the usual test reagents.[10]

Bacteriologic observations, confirmed several times on all our strains, showed that the organisms recovered from the horses, ewes and the calf were identical. Such minor variations in intensity as occurred were not referable to differences in host. For comparison we studied a strain of B. pseudotuberculosis-ovis supplied by the American Museum of Natural History. It differed markedly from ours in the formation of an orange pigment, a moist, non-friable growth, vigorous reduction of nitrates to nitrites, and non-pathogenicity for guinea-pigs. We have placed in the American Museum of Natural History cultures from our Cases IV, V, and XII for permanent reference.

Serum Tests.—Agglutination tests failed because of the rapid, spontaneous agglutination of the bacilli in 0.85% NaCl solution. Sera secured from infected horses, as well as from artificially immunized rabbits, were tried.

Hemolysis of Blood Agar Plates.—All our strains destroyed the opacity of agar plates containing 7.5% fresh, defibrinated rabbit or horse blood. Fermentable carbohydrates in the agar inhibited the lysis, as first shown by Ruediger[11] for streptococci and other organisms, and gave rise to a green pigmentation in and about the colony. This reminded us at first of sulfohemoglobin, as mentioned by Abderhalden,[12] but tests of several strains for liberation of sulfids by the method of Darling[13] resulted negatively, altho growth occurred in the first subplant from blood agar on plain agar containing 1% bismuth subnitrate. Incidentally, we confirmed the blackening of this medium by several organisms, particularly B. coli and B. proteus.

It is well known that fermentable carbohydrates in culture media protect the protein from attack and that the alkalinity of cultures is usually due to the liberation of ammonia from split protein, so that we now believe the lysis of corpuscles by certain bacteria to depend on the formation of alkali, which may determine the action of a hemolytic enzyme. But the idea that alkali alone may be responsible is supported by the fact that a drop of N/20 NaOH or NH_4OH upon a blood agar plate produces a similar hemolysis, while a drop of N/20 HCl produces green coloration analogous to that obtained by the growth of bacteria on blood agar containing fermentable carbohydrates. The differentiation of certain bacteria on the basis of hemolysis may be no more reliable, therefore, than by the use of carbohydrate media.

We have found supernatant fluid from centrifugated week-old cultures in broth (2% Witte's peptone, 0.5% NaCl, veal infusion, + 1)

[10] Jordan, General Bacteriology, 1908, p. 34.
[11] Jour. Infect. Dis., 1906, 3, p. 663.
[12] Textbook of Physiol. Chem., 1908, p. 501.
[13] Amer. Jour. Pub. Health, 1913, 3, p. 233.

slightly hemolytic for washed guinea-pig corpuscles and this hemolytic property is not destroyed by boiling for 10 minutes, a fact which suggests that for this organism hemolysis may be independent of enzyme-formation. A representative experiment follows.

B. Preisz-Nocard II was inoculated October 6, into 500 c.c. of the aforementioned broth in a 2-liter flask. October 9, a slight pellicle rapidly overspread the surface, and thickened as did that of the control, B. diphtheriae (Park and Williams, 8). October 14, the culture was centrifugated and the supernatant fluid decanted. No preservative was added. Normal, defibrinated guinea-pig blood was diluted 1 : 10 in 0.85% NaCl. From a portion the serum was removed by triple centrifugation and decantation.

A mixture of the washed guinea-pig corpuscles diluted 1:10 (1 c.c.) and the filtrate described, boiled and unboiled, after 16 hours' incubation, gave slight hemolysis. A control mixture of uninoculated broth of the same lot (1c.c.) with washed corpuscles diluted 1 : 10 (1 c.c.), gave no hemolysis.

Some of our attempts to secure a soluble hemolysin have been unsuccessful, tho the strains used had in no case lost their hemolytic action on blood agar plates.

PATHOGENICITY

Nocard and Leclainche[14] state that B. Preisz-Nocard is pathogenic for horse, ass, mule, sheep, goat, dog, rabbit, and guinea-pig. Pigeons and fowls are refractory. Our work has been confined mostly to guinea-pigs. In addition to B. mallei, as causing orchitis in guinea-pigs, Wade[15] mentions B. pyocyaneus, Bonner[16] B. coli communis, and Ramon[17] the bacillus of Malassez and Vignal; therefore, little weight can be attached to orchitis as a diagnostic sign without cultural study of the organism. But the regularity with which orchitis follows intraperitoneal injection of small amounts of B. Preisz-Nocard cultures is interesting. Table 2 shows the outcome of pathogenicity tests with several strains.

Cultures in tubes of 1% dextrose broth were incubated for 48 hours at 37 C. The granular deposit was shaken thoroughly and 1 c.c. injected into male guinea-pigs, all in the same cage. For the subcutaneous injections, animals of lighter weight were purposely chosen.

The animals comprising this series might be considered in 3 groups, according to their behavior after injection and appearance at autopsy.

[14] Les Maladies Microbiennes, 1903, 11, p. 166.
[15] Jour. Infect. Dis., 1913, 12, p. 7.
[16] Lancet, 1913, 185, p. 996.
[17] Ann. de l'Inst. Pasteur, 1914, 28, p. 585.

Guinea-pigs 97, 99, and 101: Subcutaneous tissues of the abdomen edematous; viscera normal, except for slightly hyperemic suprarenal glands, as in guinea-pigs inoculated with a toxic diphtheria culture.

Guinea-pigs 98, 100, 102, and 104: Diffuse peritonitis with little or no subcutaneous edema; highly inflamed suprarenal glands.

Guinea-pig 103: Extreme systemic intoxication on the first, second, and third days; a reduction of 20% in weight the first week. June 17, weight increased to 550 grams; soft abscess 2 cm. in diameter at site of inoculation. June 20, abscess partially reabsorbed; severe orchitis beginning. June 23, both testicles discharging creamy, caseous pus through scrotal walls. July 24, recovery complete.

TABLE 2

PATHOGENICITY OF B. PREISZ-NOCARD FOR MALE GUINEA-PIGS

Case from Which Culture Was Taken	Subcutaneous Injection					Intraperitoneal Injection				
	Guinea-pig		Orchitis	Death	Culture* at Autopsy	Guinea-pig		Orchitis	Death	Culture† at Autopsy
	Number	Weight in Grams				Number	Weight			
Horse III	97	580	None	30 hr.	None	98	740	Slight	18 hr.	Mesentery only
Horse IV	99	430	None	30 hr.	None	100	550	Slight	18 hr.	Mesentery only
Horse I	101	560	None	40 hr.	Liver abscess only	102	570	Slight	18 hr.	Mesentery only
Ox VII	103	600	Chronic severe	Very sick Recovered		104	630	Slight	40 hr.	Mesentery only

* Heart blood and subcutaneous lymph streaked on blood agar.
† Heart blood, peritoneal fluid, and peritesticular fluid streaked on blood agar.
Heart blood cultures were negative in all cases.

This variety in pathologic changes is better shown in the next series,—injected to determine the minimal lethal dose of Strain III. The results are given in Table 3.

The 24-hour growth of a cooked blood agar slant in a tube providing a slanted surface, 2.5 cm. by 6.5 cm., was removed in 10 c.c. of sterile 0.85% NaCl and ground in a mortar to separate the clumps. Dilutions of the agitated suspension were made so that 1 c.c. contained the amount injected intraperitoneally into the guinea-pigs and correspondingly plated on plain agar.

The large doses resulted in symptoms suggestive of intoxication, followed rapidly by death,—a pathologic picture simulating roughly that of guinea-pigs killed by diphtheria toxin. In these and other cases the suprarenal glands seemed to become hyperemic, particularly when death was due to intoxication rather than to the formation of abscesses. Smaller doses failed to intoxicate, but led to emasculation through, first, periorchitis, and finally, invasion of the testicles themselves.

Following the establishment of drainage from such orchitic abscesses, apparent recovery occurred. These experiments indicate the value of small intraperitoneal doses in demonstrating orchitis due to this organism.

We have also studied the production of orchitis in guinea-pigs injected with small doses of the organisms isolated from sheep, Cases IX and XII.

TABLE 3

RESULT OF DECREASING INTRAPERITONEAL DOSES OF B. PREISZ-NOCARD

Amount of Emulsion Injected and Plated c.c.	Colonies on Agar Plate (37 C. for 72 hr.)	Guinea-pig		Orchitis*	Other Symptoms	Death	Subcultures
		Number	Weight in Grams				
1.0	Uncountable	108	750	Marked	Intoxication	48 hr.	Heart blood — Peritoneum + Testicle —
0.1	About 700 ?	109	570	None	Intoxication	48 hr.	Heart blood — Peritoneum + Vas deferens +
0.01	77	110	400	Developing (3) Severe (7) Ulcerative for 3 weeks	Intoxication first few days	Fully recovered (?), but emasculated	
0.001	0	111	680	Marked (6) Ulcerative for 3 weeks	No intoxication; abscess on ankle	Fully recovered (?), but emasculated	
0 0001	0	112	510	Developing (8) Marked (10) Chronic for 3.5 months	No intoxication	Never fully recovered Used for other experiments	

* Figures indicate day of observation following injection.

Forty-eight-hour dextrose broth cultures were injected intraperitoneally in the dosage indicated in Table 4. In each case the volume injected was 1 c.c.

In this series none of the doses was large enough to cause acute intoxication; all the animals suffered from a chronic course of disease, from which, however, none recovered. This severer outcome of the chronic disease probably was due to adverse temperature conditions that did not obtain with those of the previous series; for certain guinea-pigs, apparently recovered from other experiments, died during the cold weather at this time, B. Preisz-Nocard being recovered in pure culture from their visceral lesions.

Some of the lesions noted in this series deserve special mention.

Thus, the apparently normal appearance of Guinea-pig 168 up to the day of death was probably due to an error in infection such that the inoculum failed to reach the peritoneal cavity, passing only through the abdominal

muscles to lodge beneath the parietal peritoneum. There an extensive cohesion started, which, involving the neighboring viscera, finally caused death by occlusion of the intestine. The abscess was strictly localized, the disease showing no evidence of metastasis in this animal.

Guinea-pig 169, on the other hand, developed numerous metastases throughout the lymphatics, with abscesses in the pectoral muscles, on the spleen, and particularly in the omentum. This is one of the few instances in which we recovered a culture from the heart blood. The testicles were destroyed, the tunica vaginalis containing at autopsy a mass of pus only.

In Guinea-pig 171, death was apparently due to a hemorrhage of the portal vein, which had been weakened by a substernal abscess, caused, according to cultures therefrom, by the aurococcus. The usual lesions in the peritoneum

TABLE 4

PATHOGENICITY FOR GUINEA-PIGS OF B. PREISZ-NOCARD ISOLATED FROM SHEEP

Case from Which Culture Was Taken	Dose Injected c.c.	Guinea-pig		Orchitis*	Other Symptoms	Death	Subcultures
		Number	Weight in Grams				
IX	0.1	163	530	Severe (3)	Local abscess at site of inoculation. Weight at death 410 grams	16 days	Heart blood — Omentum + Tunica vaginalis +
	0.01	167	670	Slight (14) Marked (21)	.....	30 days	Heart blood — Omentum + Testicles +
	0.001	168	350	None	Apparently normal, except for slight local induration at site of inoculation	21 days, suddenly	Heart blood — Peritoneal abscess +
XII	0.1	169	670	Marked (3) Severe (10)	Local induration at site of inoculation	15 days	Pectoral abscess + Heart blood+ Testicle + Omentum +
	0.01	170	450	Slight (3) Marked (6)	Local induration at site of inoculation	23 days	Testicle + Heart blood+
	0.001	171	380	Slight (6) Marked (20)	.....	23 days	Blood from pleural hemorrhage; Aurococcus only

* Figures indicate day of observation following injection.

were present and the testicles had been destroyed. But these lesions might have been due to the staphylococcus. Only Guinea-pig 167 showed any hyperemia of the suprarenals.

Our experience in producing orchitis with the organism from sheep does not coincide with that of Norgaard and Mohler.[13] These writers noted it but once, yet the duration (8 to 15 days) of the disease and the chronic nature of the visceral lesions in their animals indicate that the dose injected (0.3 c.c. to 0.75 c.c.) was not sufficiently large in all cases to destroy the animals before the orchitis could develop. We

[13] Sixteenth Ann. Rep. Bureau of Animal Industry, 1899, p. 638.

have recently tested Strain XII, now cultivated for over a year on artificial media, by injecting 0.01 c.c. of a 4-day glucose broth culture intraperitoneally into a male guinea-pig. Distinct orchitis developed by the sixth day. Guinea-pig 168 is the only instance in which we have failed to produce orchitis by intraperitoneal injections of small quantities of B. Preisz-Nocard from sheep, and there the reason was quite evident upon autopsy. Moreover, we have found the most prominent visceral lesions in such guinea-pigs to occur, not in the liver, but rather in the omental lymph nodes and the spleen.

Intraperitoneal injection of massive doses of culturally identical diphtheroid bacilli of human origin did not produce orchitis in guinea-pigs.

SOLUBLE TOXIN

The behavior of the guinea-pigs killed with larger amounts of culture was strongly suggestive of the acute intoxication produced by diphtheria toxin, and indeed the occurrence of a similar soluble product has been affirmed by Dassonville.[19] His results were confirmed by Carré and Bigoteau,[20] with whose principal conclusions our few experiments in this direction agree. At first, however, our cultures from Cases I, IV, and VII, failed to produce filtrates toxic for guinea-pigs, even in doses of 10 c.c., altho B. diphtheriae (Park-Williams No. 8), utilized simultaneously as a control under similar conditions, produced a filtrate containing more than 100 M.L.D. per cubic centimeter. Yet excellent surface growth, indistinguishable from that of B. diphtheriae, resulted in each flask; hence, in the light of our later knowledge, we attribute the failure to Berkefeld filtration after addition of tricresol.

Supporting the idea of a soluble toxin is the fact that week-old cultures of B. Preisz-Nocard from Case III in broth (2% Witte's peptone, 0.5% NaCl in veal infusion, + 1) proved fatally toxic in a dose of 1 c.c., but the bacilli washed 4 successive times in 0.85% NaCl or broth failed to induce intoxication in a similar dose. One cubic centimeter of the supernatant fluid of a centrifugated culture, however, while not entirely free from living bacilli, yet certainly containing a fraction only of the number injected in the washed sediment, was found to intoxicate fatally in a manner similar to that of the unaltered culture. Recognizing a weakness in these observations, we repeated this experi-

[19] Bull. de la Soc. centr. de Méd. vétérin, 1907, 84, p. 576. Jour. Comp. Path. and Therap., 1908, 21, p. 181.

[20] Rev. gén. de Méd. vétérin., 1908, 11, p. 127.

ment using Strain II, with the added precaution of Berkefeld filtration of the supernatant fluid, secured by centrifugation as follows:

October 6, a 2-liter flask of 500 c.c. veal broth (2% Witte's peptone, 0.5% NaCl, reaction + 0.5) was inoculated with B. Preisz-Nocard (Case II) and incubated at 37 C. There was a granular deposit for 2 days, giving way on the third to a thin pellicle, which became heavier from the fifth to the eighth day. A flask of the same broth inoculated with B. diphtheriae presented a precisely similar appearance from day to day and yielded, when filtered, a toxin containing from 25 to 50 M.L.D. per cubic centimeter.

October 14, the culture of B. Preisz-Nocard was removed, a part centrifugated, and one portion of the sediment washed 4 successive times in 0.85% NaCl, while another portion was similarly treated in uninoculated broth.

Table 5 gives the results of the experiment demonstrating a soluble toxin.

TABLE 5

RESULTS OF EXPERIMENT DEMONSTRATING A SOLUBLE TOXIN IN CULTURES OF B. PREISZ-NOCARD

Material Inoculated	Guinea-pig Weight in Grams	Subcutaneous Injection in c.c.	Result	Autopsy	Cultures
Unaltered culture of B. Preisz-Nocard	480	1	After 15 hr., markedly intoxicated. After 24 hr., dying	Hemorrhagic infiltration at site of inoculation. Lungs slightly hyperemic. Slight orchitis on one side	Subcutaneous tissue at site of inoculation, heart blood, and testicle sterile
Sediment washed in salt solution + 0.85% NaCl to volume of original culture	360	1	After 40 hr., slightly ill. On fourth day, local suppurative abscess at site of inoculation. Recovered		
Sediment washed in broth made up to volume with broth	...	1	Same course as that above, except for precrural abscess. Recovered		
Berkefeld filtered supernatant fluid *	440	10	Dead in 15 hr.	Slight hyperemia at site of inoculation. Lungs slightly inflamed. Peritoneal surfaces markedly hemorrhagic with red serum in cavity	Subcutaneous tissue, heart blood, and peritoneum sterile
	360	1	Markedly intoxicated; dead after 22 hr.	Slight edema at site of inoculation	Subcutaneous tissue and heart blood sterile
	360	1 (1:10 dilution in 0.85% NaCl)	No symptoms		

* No preservative added to this fluid before Berkefeld infiltration. Tested for sterility by inoculation of 5 c.c. into blood agar, 4 days' incubation.

The essentials of this experiment were corroborated with Strain III with even more convincing results. In this instance 1 c.c.

of culture, washed free of toxin by 4 successive centrifugations and decantations, failed to intoxicate acutely a guinea-pig, tho it proved fatal in 28 days. A 0.1-c.c. dose of unaltered culture proved fatal in about 48 hours, with marked intoxication and the usual appearance at autopsy. The supernatant fluid was fatally toxic in 24 hoiurs for a guinea-pig injected intraperitoneally with 1 c.c., but 0.1 c.c. failed to kill another one similarly inoculated.

Our experiments leave no doubt as to the formation of a weak soluble toxin by B. Preisz-Nocard. The strongest toxin we have secured was by centrifugation of a culture to which 0.4% tricresol had been added. This certainly renders the supernatant fluid sterile, as we have found by cultural test. Such a toxin, prepared from Strain XV, sufficed in a dose of 0.2 c.c. to intoxicate fatally a 220-gram guinea-pig in less than 48 hours. The toxicity deteriorates rapidly, however, even in the ice-chest, for we found 3 months later that 0.5 c.c. was non-fatal, tho 1 c.c. still proved lethal.

We believe that the toxin and the hemyolsin are not identical, because most of our toxic filtrates were non-hemolytic; moreover, the toxicity was destroyed by 5 minutes' boiling, whereas the hemolytic properties of certain supernatant fluids were not injured by boiling.

ANTITOXIC IMMUNITY

Antitoxin effective experimentally in a dose of 1 c.c. against 3 c.c. of toxin when tested upon guinea-pigs, was prepared by Carré and Bigoteau,[20] but it was incapable of preventing the appearance of abscesses or arresting the toxic affection of lambs known in France as "eaux rousses"; they also found sheep—well immunized against the toxin—not immune to pyogenic infection by the bacillus. Of unique interest from an academic viewpoint is a single case mentioned by Valleé[21] as quickly aborted by the use of diphtheria antitoxin. In view of the ready spontaneous healing of lymphangitic ulcers properly drained, this declaration might pass unheeded were it not supported by the careful experimentation which led Dassonville[19] to the conclusion that "le sérum anti-diphtérique paralyse l'action de la toxine du bacille de Preisz-Nocard; il en retarde considerablement les effets, parfois d'une façon indéfinie." Dassonville found that to render one fatal dose of the toxin of B. Preisz-Nocard inactive, more than 250 times as much diphtheria antitoxin was required as for one fatal dose of diphtheria

[21] Bull. d la Soc. centr. de Méd. vétérin, 1907, 84, p. 181.

toxin. In other words, it is claimed that while the toxin of B. Preisz-Nocard resembles that of diphtheria, it is not identical with it, and that the neutralization thereof by diphtheria antitoxin is partial only.

We might cite a number of experiments demonstrating the partial neutralization of B. Preisz-Nocard toxin by diphtheria antitoxin, but we would rather withhold positive statements regarding this matter for more complete proof. We did not study the action of serum from non-immunized horses upon B. Preisz-Nocard toxin, but we might recall that Dassonville[19] failed to find any evidence of protection thereby. An aggravating element in such a study might be the rapid attenuation of the comparatively weak toxin of the bacillus of Preisz-Nocard which we have experienced.

BACTERIAL IMMUNITY

Having a number of guinea-pigs which had survived previous experiments, we were interested in making tests of their resistance to further infection. Thus of 4 male guinea-pigs, weighing from 440 to 700 grams, which several weeks before had received 10 c.c., 0.1 c.c., and 0.02 c.c., respectively, of B. Preisz-Nocard III filtrate without perceptible results, 3 died within 48 hours when injected subcutaneously with 1 c.c. of a 3-day culture of this strain in glycerin broth. Pure cultures were recovered from the subcutaneous tissues, but heart blood gave no growth upon blood agar. A fourth guinea-pig was also intoxicated but recovered to suffer from a localized subcutaneous abscess, which soon healed completely.

Four other guinea-pigs were injected subcutaneously with 1 c.c. of the same culture at the same time, all having received previously cultures or emulsions of bacilli ranging from 0.001 c.c. to 1 c.c., and 3 had apparently recovered from suppurative lesions involving for 2 of them complete emasculation. The third, Guinea-pig 124, a female, had received 1000 units of diphtheria antitoxin with the original infection and had displayed only a local abscess of slight severity. The fourth animal had become emasculated and had still a swollen precrural gland. In none of these animals did the new injection cause acute intoxication, but all developed the local subcutaneous edema characteristic of animals injected with the toxin of B. diphtheriae and of B. Preisz-Nocard. The two heaviest recovered easily within 15 days. The lightest one, Guinea-pig 124, suffered for over 4 weeks from localized and metastatic abscesses, while the heavier one had the existing swelling in the precrural gland rapidly exacerbated to the point of suppuration, in addition

to the formation of an abscess at the site of inoculation. It had apparently recovered, however, within one month after the inoculation.

A further immunity test was made upon these guinea-pigs by injecting them intraperitoneally, together with 3 others, of which 2 still suffered from suppurative processes, with 5 c.c. of a 7-day glucose broth culture of B. Preisz-Nocard II. This test, a severe one, intoxicated all of the animals under observation, except Guinea-pig 124, and killed acutely some of those which might, from their previous resistance, have been considered quite immune. Others apparently recovered, but in certain instances only after a severe course of recurrent abscesses, particularly of the testicular remnants or of the lymphatic nodes.

The findings at autopsy in some of these animals are worthy of special mention.

Thus, Guinea-pigs 110, 112, and 117, comprising those acutely and fatally intoxicated, showed in each case a hemorrhagic condition of the lungs; a diffuse, purulent peritonitis, particularly localizing in and invading the spleen; and a marked hyperemia and enlargement of the suprarenal capsules.

Guinea-pigs 103 and 111, apparently on the way to recovery, died unexpectedly during a sharp, frosty spell of weather. The lungs of the former were hemorrhagic and the suprarenals were hyperemic, but the only pus discoverable was in the testicular vestiges and this contained B. Preisz-Nocard in pure culture. Both animals were emaciated, particularly Guinea-pig 111. In the latter there were practically complete destruction of the spleen, as well as the testicles, and deep congestion of the suprarenal capsules, which were enlarged nearly to the size of the kidneys.

These observations show that a variable degree of immunity is conferred by an attack of lymphangitis in guinea-pigs. In the practical immunization of large animals effort should be directed toward antitoxic, as well as bacterial, immunity. The latter seems to us, however, to be more important in ulcerative lymphangitis than in either diphtheria or tetanus.

SUMMARY

We have isolated B. Preisz-Nocard from characteristic abscesses in 11 horses and one calf. The etiology of the lesions from which it was obtained is identical with that of caseous lymphadenitis of sheep, and the disease in horses known as ulcerative lymphangitis should be differentiated by laboratory diagnosis from farcy, epizootic lymphangitis, and sporotrichosis, all of which have a mutual resemblance clinically.

B. Preisz-Nocard is a diphtheroid bacillus, presenting interesting characteristics as follows: (1) the production of orchitis in guinea-pigs, as well as suppurative processes generally throughout the lymphatics; (2) the hemolysis of blood agar plates not containing an excess of fermentable carbohydrate; (3) the elaboration of a soluble toxin, resembling but not identical with that of diphtheria, yet being neutralized partly by diphtheria antitoxin. This apparent partial neutralization suggests the existence of group reactions among soluble bacterial toxins analogous to the group reaction of precipitins and agglutinins.

We again draw attention to the uncertainty of experimental orchitis in guinea-pigs as a certain test for glanders, and emphasize the necessity of microscopic and cultural examination of pus from such lesions for diagnostic purposes.